

## UVssDNA Antibody (Clone C3B6)

**Cat# 4350-MC-100**

**Antibody for the detection of (6-4)-  
dipyrimidine photoproducts by ELISA and  
indirect immunofluorescence**

**100 µg**

**UVssDNA Antibody (Clone C3B6)**

**Cat# 4350-MC-100**

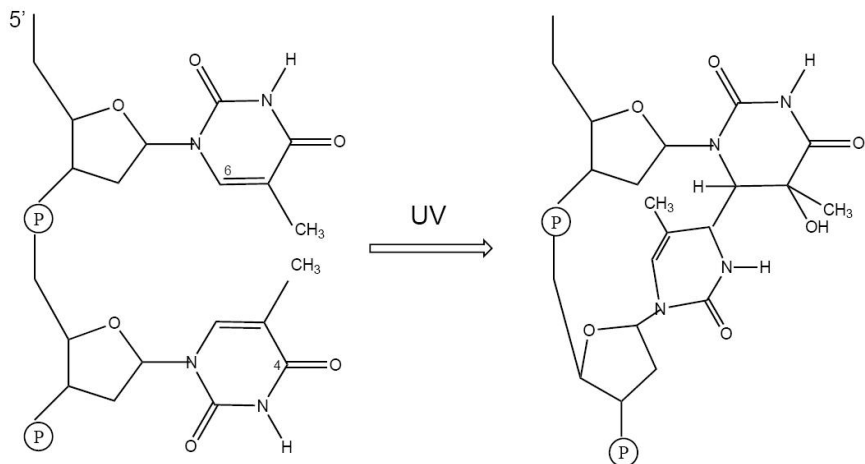
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## I. Introduction

Immunological assays for DNA photoproducts provide a rapid means to quantitate photodamage in DNA. Photochemical damage is considered to be an important factor in the development of skin cancer. Sensitive immunological methods that detect individual DNA adducts facilitate the study of the biological significance of DNA lesions.

Trevigen's monoclonal UVssDNA antibody (clone C3B6) (cat# 4350-MC-100) detects (6-4)-dipyrimidine photoproducts. Specificity has been demonstrated for (6-4)-dithymidine, whereas the (6-4)-dicytidines or cyclobutadipyrimidines are not recognized. A low level of cross reactivity was observed with repeating (6-4)-thymine/cytidines within an oligonucleotide sequence. The UVssDNA clone C3B6 antibody recognizes (6-4)-dipyrimidines in single stranded DNA at least 4 nucleotides long.



**Figure 1:** Structure of 6,4',5', methylpyrimidin-2', one'-thymine--an example of a (6-4)-photoproduct. Linkage occurs between the C-6 position of one thymine and the C-4 position of the adjacent thymine. The plane of the 3' pyrimidine is shifted 90° relative to the 5' thymine and introduces a major distortion to the DNA helix.

The antibody may be used in enzyme linked immunosorbant assay (ELISA) for indirect quantitation of UV damage and in immunofluorescent assay of tissues and cells. Protocols are provided for competitive ELISA and for the detection by immunofluorescence of (6-4)-dithymidine in individual cells and fresh frozen tissues.

## II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.

## III. Materials Supplied

<u>Catalog Number</u>	<u>Component</u>	<u>Amount Provided</u>	<u>Storage Temperature</u>
4350-MC-100	UVssDNA Antibody (clone C3B6)	100 µg	-20 °C

## IV. Materials/Equipment Required But Not Supplied\*

\* Please see the reagent list for each of the methods provided (below).

### Disposables:

1. 1 - 200 µl and 100 - 1000 µl pipette tips
2. 0.5 and 1.5 ml microtubes
3. 15 ml conical (adherent and suspension cell preparation)
4. 50 ml conical (tissue preparation)
5. Microtiter Plates

### Equipment:

1. Micropipettes
2. Multichannel pipettor 1 - 50 µl and 50 µl - 200 µl
3. UV light source
4. 96-well plate reader with 450 nm filters (capable of taking readings every minute for ten minutes and exporting data to Excel spreadsheet)
5. Centrifuge for processing samples

## V. Inhibitor ELISA assay

Trevigen's anti-UVssDNA antibody (cat# 4350-MC-100) is ideal for determining damage to DNA using an ELISA. Test DNA may be purified from cells and tissues using a variety of standard techniques or commercially available kits. (6-4)-photoproducts are labile in strong alkaline solutions. The following indirect ELISA protocol was developed to detect T<>T lesions in small amounts of DNA.

### Reagents and materials needed:

10X PBS (cat# 4870-500-6)	test DNA
bovine serum albumin (BSA)	UV light source
native calf thymus DNA (cat# 9600-5-D)	microtiter plates
secondary antibody conjugate and detection system of choice (e.g. anti-mouse horseradish peroxidase and TACS-Sapphire™ reagent*)	

\* TACS-Sapphire (cat# 4822-96-08)

### Method:

1. Prepare a solution of calf thymus DNA in 0.1X PBS at 1 mg/ml. Boil for 10 minutes in a boiling water bath and then chill rapidly on ice. Irradiate the DNA at 1000 J/m<sup>2</sup> using ultraviolet light (254 nm). Store at -20°C in small aliquots.
2. Add 100 µl of a 50 ng/ml solution of denatured calf thymus DNA irradiated at 1000 J/m<sup>2</sup> (prepared in step 1) in 0.1X PBS to the wells of a microtiter plate (select a plate brand and style appropriate for ELISA and the detection method of choice. Add 1X PBS to the no antigen control wells (no DNA at the bottom).
3. Dry the plates in an oven or vented incubator at 34°C (not 37°C) for 2 days or until dry (plates may then be stored wrapped at -20°C for several months).

4. Add 0.2X PBS, 1% BSA, 15 ng/ml calf thymus DNA, to each well and incubate at 37°C for 1 hour. Note that addition of calf thymus DNA is optional and may be omitted if background is low.
5. Wash each well four times with 0.2X PBS.
6. Dilute antibody between 1:10,000 and 1:100,000 in 0.2X PBS, 1% BSA and warm to 37°C.
7. Heat denature test sample DNA, or inhibitor DNA for standard inhibition curve. Prepare the DNA at 0.6 µg/ml in 0.2X PBS, 1% BSA. Genomic DNA should be sheared by passing through a 26 gauge needle 10 times before denaturation.
8. Prepare the following samples:
  - a) Wells without antigen (no dried DNA in well, add inhibitor DNA/antibody mixture described in d) (E<sub>3</sub> wells).
  - b) Wells without inhibitor: add diluted antibody only (E<sub>2</sub> wells).
  - c) Wells without primary antibody: Add 0.2X PBS, 1% BSA without primary antibody.
  - d) Combine equal amounts of standard inhibitor DNA or test DNA with diluted antibody and add 100 µl per well (E<sub>1</sub> wells). Incubate plate for 30 minutes at 37 °C.
9. Wash 4 times with 0.2X PBS, 1% BSA, 0.05% Tween<sup>®</sup> 20.
10. Proceed with antibody detection method of choice e.g. anti-mouse horse-radish peroxidase with color development using TACS-Sapphire, specifically designed for quantitative readout in ELISA and other plate based assays.

Depending upon the microtiter plate, the detection method, and the quality of reagents, the actual conditions may vary. Optimization of primary and secondary antibody dilutions may be required.

#### Interpretation of data:

$$\text{percent inhibition} = 100 \times (E_2 - E_1) / (E_2 - E_3)$$

where E<sub>1</sub> is the average OD of the wells containing inhibitor  
 E<sub>2</sub> is the average of OD of the wells without inhibitor (0%) inhibition  
 E<sub>3</sub> is the average of OD of the wells without antigen (100% inhibition)

The percentage of inhibition obtained with the standards can be translated into number of T<->T present in the DNA to quantitate the damage present in the experimental samples.

**Note: approximately 0.007 T<->T lesions are expected per kilobase of UV 254 nm treated DNA, create a standard curve in the range of damage that is appropriate to your samples.**

## VI. Immunoassay of intact cells and tissues

Required reagent for tissues: Tissue-Tek<sup>®</sup> OCT compound

Trevigen's Anti-UVssDNA antibody may be used to directly visualize DNA damage in cells allowing for demonstration of DNA damage in conjunction with tissue localization. The following protocol provides the basic details for using this antibody *in situ*. Conditions may have to be optimized depending upon the cell or tissue type and on the quality of reagents used. Details are provided for sample preparation and fixation, pretreatments, and antibody binding and detection.

### 1. Sample Preparation a) Preparation of cells

#### Preparation of Suspension Cells

Cells grown in suspension can be fixed in solution and then spotted onto pretreated glass microscope slides for processing. This method is quick and easy and requires no special equipment.

Method:

1. Harvest cell suspension by centrifugation at 500 x g for 5 minutes at room temperature.
2. Discard media and resuspend cells in 5 ml 100% methanol prechilled to -20°C. Let stand for 5 minutes on ice.
3. Harvest cells by centrifugation at 1000 x g for 5 minutes at 4 °C and discard methanol.
4. Resuspend cells in 5 ml 100% acetone prechilled to -20°C. Let stand on ice for 5 minutes.
5. Spot 1 x 10<sup>5</sup> cells onto a clean glass microscope slide. Air dry for 10 minutes at room temperature.

**Note: Glass slides pretreated for electrostatic adherence are recommended (cat# 4861-100). Other pretreatments (e.g. gelatin) can cause increased background staining.**

6. Immerse in 70% ethanol for 10 minutes and then air dry overnight at room temperature or dry at 45°C for 2 hours.
7. Immerse in 1X PBS and proceed to Section 2, Pretreatment.

#### Preparation of monolayer cells

##### Sterile Chamber Slides

For optimal outcomes, cells should be grown on a surface that allows for both fixation and direct labeling such as sterile chamber slides. Remove the chamber wells and gasket after fixation.

##### Sterile Slides

Other cell culture methods include culturing directly on microscope slides. The slides must be sterile and, if necessary, pretreated to ensure cell adhesion. Sterilize microscope slides by autoclaving in a large glass Petri dish. If

needed, coat slides with sterile poly-L-lysine or collagen. Place sterile microscope slides in culture vessel directly before plating cells.

#### Sterile Glass Cover Slips

Cells can be cultured directly on sterile cover slips that are placed into a 12 or 24-well tissue culture plate. Sterilize cover slips by autoclaving in a large glass Petri dish. If needed, coat cover slips with sterile poly-L-lysine or collagen. Place sterile glass cover slips in wells of tissue culture dishes (12 mm cover slips fit into 24 well tissue culture plates) using fine tipped sterile forceps. Handle only at edges prior to cell plating.

#### Method:

1. Remove media from cells and rinse once with 1X PBS at room temperature.
2. Fix cells for 5 minutes on ice with 100% methanol prechilled to -20°C.
3. Remove coverslips and immerse in 100% acetone prechilled to -20°C in a glass petri dish for 5 minutes. (Note: Acetone and plastic are not compatible.)
4. Remove cover slips. Allow to air dry.
5. Proceed to section 2, pretreatments.

#### b) Preparation of tissues

For immunostaining, use fresh frozen tissue. Rapidly freeze tissue upon biopsy (immediately after removal) by immersing in liquid nitrogen or on dry ice in Tissue-Tek OCT compound. Store frozen tissue below -70°C. Position the sample within the cutting matrix in a suitable container. Immerse embedded tissue in isopentane chilled on dry ice. Using the cutting matrix, attach the sample to cutting block. Equilibrate the temperature of the cryostat before sectioning. Collect sections between 6-15 µm on glass slides pretreated for electrostatic adherence.

1. Dry overnight at room temperature or for at least 2 hours at 45°C on a slide warmer.
2. Immerse for 5 minutes in 100% methanol prechilled to -20°C.
3. Immerse for 5 minutes in 100% acetone prechilled to -20°C
4. Air dry.
5. Proceed with labeling.

**Many samples are routinely fixed by immersion or perfusion methods, however, the utility of the Anti-UVssDNA antibody for tissues fixed in cross-linking fixatives and embedded in paraffin has not yet been determined.**

#### 2. Pretreatment for immunostaining

The Anti-UVssDNA Antibody detects (6-4)-dipyrimidine photoproducts in denatured DNA. Pretreatments are required to permeabilize the sample for protein and antibody access, to remove RNA (which may also contain UV photoproducts), and to denature the DNA prior to antibody addition. The conditions described below for indirect immunofluorescence are provided as a guide only. The actual times and temperatures of incubation may require optimization.

#### Reagents required:

hydrochloric acid	10X PBS
sodium chloride	Tris/EDTA (TE)
sodium hydroxide	ethanol
formaldehyde	proteinase K
RNase	sodium citrate

1. Treat fixed and immobilized sample with 0.05 N HCl for 5 minutes on ice.
  2. Wash three times with 1X PBS.
  3. Incubate in 100 µg/ml RNase in 150 mM NaCl, 15 mM sodium citrate for 1 hour at 37°C.
  4. Wash sequentially in 1X PBS, 35% ethanol, 50% ethanol, and 75% ethanol, for 2 minutes each.
  5. Denature DNA in situ with 0.15 N NaOH in 70% ethanol for 2 - 4 minutes.
  6. Wash sequentially in 70% ethanol (containing 4% v/v formaldehyde), 50% ethanol, 35% ethanol, and 1X PBS for 2 minutes each.
  7. Incubate in 5 µg/ml proteinase K in 20 mM Tris, 1 mM EDTA, pH 7.5 (TE) for 10 minutes at 37°C.
  8. Wash several times in 1X PBS.
- Note: Steps 3 and 7 are optional but optimal signal to noise ration has been obtained using these reagents.

#### 3. Antibody binding and detection

The actual dilutions of antibody, and times and temperatures of incubations may require optimization.

#### Reagents required:

10X PBS	dithiothreitol
BSA	deionized water
FITC-labeled anti-mouse antibody	glycerol
Tween <sup>®</sup> 20	propidium iodide

1. Dilute the antibody to 6 µg/ml in 1X PBS, 1% BSA, 0.01% Tween<sup>®</sup> 20, on ice.
  2. Incubate pretreated sample in diluted antibody in humidified chamber overnight at 4°C.
  3. Wash several times in 0.1X PBS for 5 minutes each wash.
  4. Incubate sample in 25 µg/ml FITC-labeled anti-mouse antibody in 1X PBS, 1% BSA for 30 minutes at 37°C.
  5. Wash several times in 0.1X PBS, 0.05% Tween<sup>®</sup> 20 for 5 minutes each wash.
  6. Rinse in deionized water.
  7. Mount coverslip using 90% glycerol, 0.03 M dithiothreitol, pH 8.2.
- Note: For quantitation of total DNA the cells may be counterstained after step 5 with 10 µg/ml propidium iodide in 1X PBS for 10 minutes. Include propidium iodide (0.5 µg/ml) in the mounting solution (step 7).

## VII. References:

- 1) Liang X, Pickering MT, Cho N-H, Chang H, Volkert MR, Kowalik TF, and Jung JU (2006) Deregulation of DNA Damage Signal Transduction by Herpesvirus Latency-Associated M2. *J. Virol.* **80**:5862-74.
- 2) Strickland PT, Creasey JS. (1988) Immunoassay of dithymidine cyclobutane dimers in nanogram quantities of DNA. *IARC Sci Publ.* **89**:341-4.
- 3) Strickland PT, Nikaido O, Matsunaga T, Boyle JM. (1992) Further characterization of monoclonal antibody indicates specificity for (6-4)-dipyrimidine photoproducts. *Photochem Photobiol.* **55**:723-7.
- 4) Bruze M, Emmett EA, Creasey J, Strickland PT. (1989) Cyclobuta-dithymidine induction by solar-simulating UV radiation in human skin: II. Individual responses. *J Invest Dermatol.* **93**:341-4.
- 5) Lesko SA, Li W, Zheng G, Callahan D, Kaplan DS, Midden WR, Strickland PT. (1989) Quantitative immunofluorescence assay for cyclobutyl dithymidine dimers in individual mammalian cells. *Carcinogenesis.* **10**:641-6.

## VIII. Related Products Available From Trevigen

Contact Trevigen for details of our unique product line for studying DNA damage and repair. All of Trevigen's kits include highly qualified enzymes, substrates, buffers, full instructions for use, and a synopsis specific for your kit.

### Antibodies

Catalog #	Description	Size
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 µl
4336-BPC-100	Anti-PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg
4354-MC-050	Anti-8-oxo-dG (clone 2E2)	50 µl
4360-MC-100	Anti-BPDE (clone 8E11)	100 µg
4365-MC-100	Anti-BPDE DNA (clone 5D11)	100 µg
4410-PC-100	Anti-FEN-1 polyclonal	100 µl
4411-PC-100	Anti-γ-H2AX polyclonal	100 µl
4415-PC-100	Anti-APE/Ref-1 (clone 13B8E5C2)	100 µl
4420-PC-100	Anti-Fpg polyclonal	100 µl
4421-MC-100	Anti-XRCC1 (clone 144)	100 µg
4430-MC-100	Anti-XPB (clone CD12)	100 µg
4445-MC-100	Anti-Polymerase β (clone 61)	100 µg
4451-MC-200	Anti-Rad1 (clone 33)	200 µg

### CometAssay™ Kits:

Catalog #	Description	Size
4250-040-K	CometAssay™	40 samples
4251-050-K	CometAssay™ Silver Kit	50 samples
4254-200-K	CometAssay™ Silver Staining Kit	200 samples
4252-040-K	CometAssay™ Higher Throughput Kit	40 samples
4253-096-K	CometAssay™ Kit 96 Wells	96 samples

### Control Cells:

Catalog #	Description	Size
4256-010-CC	CometAssay™ Control Cells	10 assays

### FLARE™ Assay Kits:

Catalog #	Description	Damage Recognized	Size
4100-100-FK	UVDE Kit	Cyclobutane pyrimidine dimers, (6-4) photoproducts	75 samples
4100-100-FM			100 samples
4040-100-FK	Fpg Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4040-100-FM			100 samples
4045-01K-FK	Endonuclease III Kit	Thymine Glycol, 5,6-dihydrothymine, urea, 5-hydroxy-6-hydroxy-thymine, 5,6-dihydro-uracil, alloxan, 5-hydroxy-6-hydroxy-uracil, uracil glycol, 5-hydroxy-5-methylthio-dantoin, 5-hydroxycytosine, 5-hydroxy-uracil, methyl-tartronylurea, thymine ring saturated or fragmentation product	75 samples
4045-01K-FM			100 samples
4130-100-FK	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4130-100-FM			100 samples
4055-100-FK	T4-PDG Kit	Cis-syn isomers of cyclobutane pyrimidine dimers	75 samples
4055-100-FM			100 samples
4065-100-FK	cv-PDG Kit	Cis-syn and trans-syn isomers of cyclobutane pyrimidine dimers	75 samples
4065-100-FM			100 samples

### CometAssay™ Electrophoresis System:

Catalog #	Description	Size
4250-050-ES	CometAssay™ ES	each

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

### Trevigen, Inc.

8405 Helgerman Ct. Gaithersburg, MD 20877

Tel: 1-800-873-8443 • 301-216-2800

Fax: 301-560-4973

e-mail: [info@trevigen.com](mailto:info@trevigen.com)

[www.trevigen.com](http://www.trevigen.com)



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